CORTISOL-INDUCED CHANGES IN U.V. ABSORPTION SPECTRA OF ISOLATED NUCLEI FROM RAT LIVER AND THYMUS

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SUMMARY: Cortisol at a concentration of 5×10^{-6}M induces profound changes in U.V. absorption spectra of isolated nuclei from rat liver and thymus. The changes occur within the first 10 min of cortisol action. In both types of nuclei, a blue shift of 5-10 nm from the normal absorption maximum at 260-270 nm is evident. In addition, liver nuclei exhibit an elevation of the spectrum at 230-270 nm (increased U.V. absorption), while the spectrum of thymus nuclei becomes flattened. No such changes occur in nuclei exposed to a physiologically inactive hormone (pregnenolone). The results are interpreted as evidence for cortisol-induced perturbations in deoxyribonucleoprotein structure with consequent changes in the degree of condensation of nuclear chromatin.

Glucocorticosteroid hormones influence RNA metabolism in liver and in lymphatic tissue. Liver cells respond to corticosteroids by an increased rate of RNA synthesis (1,2). In vitro studies have revealed that the template activity of nuclear chromatin from liver cells is increased in the presence of corticosteroids (3,4). Glucocorticosteroids also induce an increase in the number of actinomycin D binding sites in DNA of chromatin of liver nuclei (4,5), a phenomenon which is believed to indicate a dissociation of DNA-protein complexes (6) related to the activation of nuclear genome (7,8). Glucorticoid-induced changes in liver nuclear chromatin occur not only *in vivo* but also *in vitro*, i.e. when isolated nuclei (5) or chromatin (4) are treated with the hormones.

Lymphoid cells, in direct contrast to liver cells, respond to glucocorticosteroids with suppression of RNA synthesis (5,9-14). Isolated thymus nuclei (5,12,13) or chromatin extracted from thymus cells exposed to the hormones *in vivo*, exhibit decreased RNA-polymerase activity (11,13) and a decrease in the number of actinomycin D binding sites (5,14). Unlike the situation with liver cells, neither isolated nuclei nor the chromatin of

lymphoid cells respond to glucocorticosteroids *in vitro* (4,5). It can be stated that in both cell types the steroid hormones seem to induce changes in nuclear chromatin, but the nature of change is possibly different in different nuclei types. Recently it has been shown that isolated nuclei can be studied by optical methods (15-17). In the present communication we describe corticosteroid-induced changes in U.V. absorption spectra of isolated nuclei from liver or thymus.

MATERIAL AND METHODS: Two-month-old Sprague-Dawley rats were decapitated, the livers and thymuses were excised and transferred into buffered salt solution at 0°C. Liver nuclei were isolated according to the procedure of Chauveau *et al.* (18) using the modification of Pogo *et al.* (19). The tissue was homogenized in 0.32M sucrose-3mM $MgCl_2$ by blending at 5,000 rpm in a Virtis homogenizer for 2 min. The suspension was filtered through eight-layers of gauze, and centrifuged at 800xg for 10 min. The crude nuclear pellet was resuspended in 2.4M sucrose-1mM $MgCl_2$ by blending at 3,000 rpm for 2 min. The suspension was centrifuged at 50,000xg for 60 min. Sedimented nuclei were resuspended in 0.25M sucrose-4mM $MgCl_2$-0.1M tris-HCl buffer, pH 7.6 (STM) and centrifuged at 1,000xg for 5 min. The nuclear pellet was washed once more in SMT and the final white pellet of liver nuclei resuspended in STM at a concentration of approximately $3x10^7$ nuclei/ml.

Thymus nuclei were isolated as described before (5), following which they were resuspended in SMT medium, at the same concentration as liver nuclei. Isolation of nuclei was always carried out at 0-4°C. The stock suspensions of nuclei remained at 0°C before being subjected to U.V. measurements.

Optical measurements were done with a Cary-15 spectrophotometer, using 1 cm (4ml volume) quartz cuvettes. Suspensions of nuclei at a concentration of 0.7 to $1.0x10^7$/ml obeyed Beer's law throughout the spectral

range employed. The measurements and incubations of nuclei with hormones were performed at room temperature.

RESULTS: Fig. 1 shows a representative absorption spectra of liver and thymus

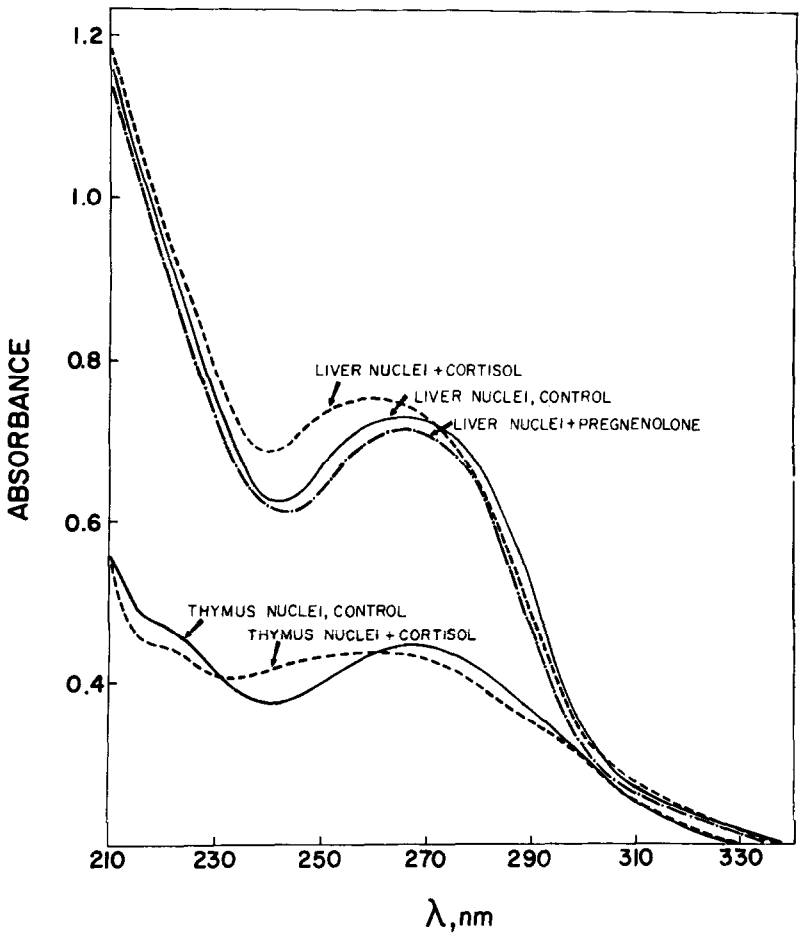

Figure 1. Absorption spectra of liver and thymus nuclei, control and steroid-treated. The U.V. absorption of liver nuclei, suspended in 0.25M sucrose-4mM $MgCl_2$-0, 1M tris-HCl at pH 7.6 (SMT) at concentration 8.0×10^6/ml was recorded using SMT solution in the reference light path (control nuclei). Then, 20 µl of concentrated solution of cortisol (Δ 4-Pregnen-11 , 17 , 21-triol-3,20-dione; Sigma, dissolved in 4% v/v ethanol in SMT) was added into the sample and the referenced cuvettes to obtain a final concentration 5×10^{-6}M. The second measurement was performed 10 min after addition of the hormone (nuclei + cortisol). The experiment on thymus nuclei was performed in a similar way, except that the nuclei were measured at concentration of 9.0×10^6/ml. In another experiment, after recording the spectrum of liver nuclei, the biologically inactive steroid (Δ 5-pregnene-3 -01-20-one) was added into the cuvettes to a final concentration of 5×10^{-6}M. The spectrum was recorded 10 min later (nuclei + pregnenelone).

nuclei alone, and after addition of steroids. The spectrum of liver nuclei closely resembles that of soluble nucleohistone (16,17). In contrast, thymus nuclei exhibit a broader band. In the presence of cortisol, both liver and thymus nuclei show a 5-10 nm shift of absorption maximum to the blue. In addition, an increase in magnitude of the band at 260 nm occurs in the case of liver nuclei; the cortisol-induced band in thymus nuclei is flattened. No such changes are observed when nuclei are treated with the biologically inactive steroid (5-pregnene-3 -ol-20-one).

We are aware that light scattering may interfere with optical measurements of nuclei suspensions. In the present studies the effect of light scattering was found to be minimal since moving the cuvettes from standard position to close to the detector did not have any appreciable effect on the spectrum. Moreover, the results were verified by the differential spectrum which is not affected by light scattering.

The differential spectrum (Fig. 2) was recorded using identical suspensions of nuclei in both sample and reference cuvettes. The magnitude of the change induced by cortisol in the different spectrum is completely consistent with the conventional absorption measurement (Fig. 1).

DISCUSSION: There is a marked difference between the U.V. absorption spectra of liver and thymus nuclei. It is believed the difference is due to the different states of chromatin condensation (16,17). The chromatin in liver nuclei is more homogenous than in thymic nuclei and has U.V. absorption properties similar to soluble nucleohistone. The more flattened spectrum observed with thymus nuclei occurs presumably because there are local areas of highly condensed chromatin within the nucleus ("granular chromatin") (17). The changes observed in the spectrum of liver or thymus nuclei following cortisol administration presumably reflect perturbations in nucleoprotein structure induced by cortisol. The maximal changes occur at concentration of 5×10^{-6} M and above of cortisol (20). The maximal response of liver or

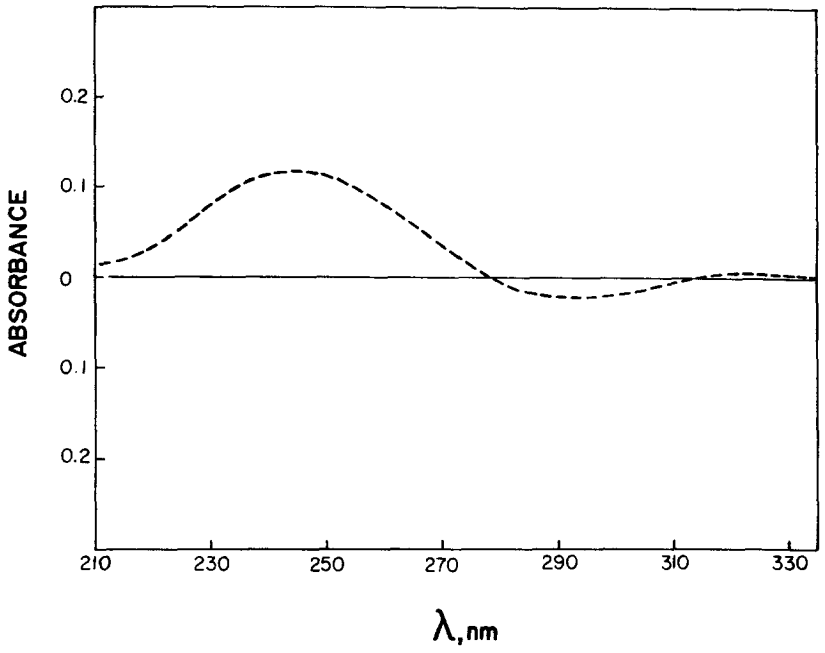

Figure 2. Different spectrum of liver nuclei treated with cortisol at $5x10^{-6}$M concentration. The measurement was performed using identical suspensions of liver nuclei ($8x10^6$/ml) in the sample and in reference cuvettes. Ten min before the measurement 20 µl of cortisol solution was added to the sample cuvette and 20 µl of solvent (4% ethanol in SMT) to the reference sample.

thymus nuclear chromatin to cortisol expressed as an alteration of actinomycin D binding properties (4,5,14) or as a change of template activity (4,5,10) occur also at $5x10^{-6}$M cortisol concentration. Considering the above, and also that biologically inactive pregnenolone does not affect U.V. spectra, we suspect that the perturbations in chromophoric portion of chromatin in the presence of cortisol may represent changes in nucleoprotein related to the biological function of the hormone. Experiments to test this hypothesis are in progress.

Two different types of changes in U.V. spectrum are observed as a result of cortisol action, namely, the blue shift in the absorption maximum (5-10 nm) and the flattening (thymus) or elevation (liver) of the absorption

spectra. The first type of change which was observed in nuclei of both tissues presumably indicates interaction of hormones with the chromophoric portion of nucleohistone. In view of the hydrophobic stacking properties of the bases in nucleic acids, it is not surprising that hydrophobic molecules of biological importance such as steroid hormones or polycyclic carcinogens interact strongly with nucleic acids (21,22). This interaction, however, is associated with a conformational perturbation of supercoiled nucleohistone, possibly due to an interaction in the chemical environment of the chromophore. The flattening of absorption spectrum of thymus nuclei as a result of cortisol action seems to represent the morphological changes of the type of increased "granulation" that is characteristic of the more condensed chromatin in the nuclei (17). The elevation of the spectra of liver nuclei in the presence of cortisol may indicate the increased degree of homogeneity of chromatin within the nuclei. Similar elevation of the spectra also occurs at high ionic strength and is presumed to be the sign of a dissociation of DNA-protein complex (16,17,20). The above interpretation of an elevation or flattening of spectra in the presence of cortisol remains in conformity with the observations that the degree of association of DNA-protein complexes in target tissue nuclei is affected by the appropriate hormones, as measured by the number of sites in DNA which react with actinomycin D or with basic dyes (4, 5,14,23), or by changes in thermal stability of DNA within the nuclear chromatin (24).

There are several possible sites of interaction of corticosteroid hormones with nuclear chromatin. Corticosteroids bind to histones (25-27); the reaction involves covalent binding with terminal arginine and, to a lesser extent, lysine (27). The biological significance of this interaction is uncertain, since inactive steroids react with histones as well as, or better than, the active ones (27). The interaction of steroid hormones with non-histone protein components of chromatin of appropriate target cells (e.g. progesterone - oviduct) has been also shown (28,29); the tissue specificity

of these responses point to their biological relevancy (29). Whether or not similar interactions occur in liver or thymus is not known. Steroid hormones can interact also with DNA, either directly (22,23), or indirectly, via steroid-receptor cytoplasmic macromolecules (30-32). Recent evidence suggests that this type of interaction may be biologically important in regard to modulating RNA metabolism in lymphatic (30) or in liver (hepatoma) cells (31,32).

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REFERENCES:

1. Feigelson, M., Gross, P.R., and Feigelson, P., Biochim. Biophys. Acta 55,495 (1962)
2. Kidson, C., and Kirby, K.S., Nature, 203, 559 (1964).
3. Lukacs, I., and Sekeris, C.E., Biochim. Biophys. Acta, 134, 85 (1966).
4. Beato, M., Seifart, K.H., and Sekeris, C.E., Arch. Biochem. Biophys., 138, 272 (1970).
5. Darżynkiewicz, Z., and Jacobson, B., Exp. Cell Res., 67, 49 (1971).
6. Jurkowitz, L., Arch. Biochem. Biophys., 111, 88 (1965).
7. Berlowitz, L., Pallotta, D., and Sibley, C.H., Science,164, 1527 (1969).
8. Darżynkiewicz, Z., Bolund, L., and Ringertz, N.R., Exp. Cell Res., 56, 418 (1969).
9. Kidson, C., Biochem. Biophys. Res. Comm., 21, 283 (1965).
10. Makman, M.H., Dvorkin, B., and White, A., J. Biol. Chem., 241, 1646 (1966).
11. Fox, K.E., and Gabourel, J.D., Molec. Pharmacol., 3, 479 (1967).
12. Drews, J., and Wagner, L., Europ. J. Biochem., 13, 231 (1970).
13. Makman, M.H., Nakagawe, S., Dvorkin, B., and White, A., J. Biol. Chem., 245, 2556 (1970).
14. Darżynkiewicz, Z., and Andersson, J., Exp. Cell Res., 67, 39 (1971).
15. Wagner, T.E., Biochem. Biophys. Res. Comm., 38, 890 (1970).
16. Wagner, T., and Spelsberg, T.C., Biochemistry, 10, 2599 (1971).
17. Olins, D.E., and Olins, A.L., J. Cell Biol., 53, 715 (1972).
18. Chauveau, J., Moule, Y., and Rouiller, ., Exp. Cell Res., 11, 186 (1956).
19. Pogo, A.D., Allfrey, V.G., and Mirsky, A.E., Proc. Natl. Acad. Sci. U.S., 56, 550 (1966).
20. Darżynkiewicz, Z., and Chakrabarti, B., Exp. Cell Res. (submitted).
21. Tso, P.O.P., and Lu, P., Proc. Natl. Acad. Sci., 51, 17 (1964).
22. Tso, P.O.P., and Lu, P., Proc. Natl. Acad. Sci., 51, 272 (1964).
23. Salaganik, R.I., Morozova, T.M., and Zakharov, M.A., Biochim. Biophys. Acta, 174, 755 (1969).
24. Ritzen, M., Carlsson, S.-A., and Darżynkiewicz, Z., Exp. Cell Res., 70, 462 (1972).
25. Sluyser, M., J. Mol. Biol., 22, 411 (1966).
26. Monder, C., and Walker, M.C., Biochemistry, 9, 2489 (1970).
27. Sunaga, K., and Koide, S.S., Biochem. Biophys. Res. Comm., 26, 342 (1967).
28. Bruchovsky, N., and Wilson, J.D., J. Biol. Chem., 243, 5953 (1968).

29. O'Malley, B.W., Spelsberg, T.C., Schrader, W.T., Chytil, F., and Steggles, A.W., Nature, 235, 141 (1972).
30. Rosenau, W., Baxter, J.D., Rosseau, G.G., and Tompkins, G.M., Nature New Biol., 237, 20 (1972).
31. Baxter, J.D., Rouseau, G.G., Benson, M.O., Garcea, R.L., Ito, J., and Tomkins, G.M., Proc. Natl. Acad. Sci. U.S., 69, 1892 (1972).
32. Beato, M., Brandle, W., Biesewig, D., and Sekeris, C.E., Biochim. Biophys. Acta, 208, 125 (1970).